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viewpoint

Certain promise and uncertain peril

The debate on xenotransplantation • *by Robin A. Weiss*

A Hippocratic oath is required not only for the individual patient but also for the community at large. This thought is provoked by a number of debates in health care on the potential conflict between personal need and the common good. Should high-tech medicine be funded at the expense of preventative and community health? Did the saving of individual lives through antibiotics spawn killer strains of multiple resistant bacterial pathogens? Similar concern has arisen over xenotransplantation—the grafting of animal tissues into humans.

'Uncertain peril and certain promise' was Joshua Lederberg's epithet on the emerging field of genetic engineering 25 years ago, and it aptly describes xenotransplantation today. The 'certain promise' of xenogeneic transplantation is that it could provide a supply of cells, tissues and organs to treat a number of serious human diseases. The 'uncertain peril' is the possibility of a new human epidemic emerging from transplanted animal organs, which elicited a call for a moratorium so that the ethical and safety issues involved can be more publicly debated and resolved (Bach *et al.*, 1998).

The threat is novel viruses that lurk undiscovered in animal tissues

The need for organs is pressing. Although Eurotransplant, which co-ordinates donor organs in Austria, Belgium, Germany and The Netherlands, supplied 5471 hearts, livers, kidneys, lungs and pancreases last year, 5174 patients were still on the waiting list. In the USA, nearly 70 000 patients are waiting for an available organ right now. As xenotransplantation could provide an unlimited source of cells and organs, the lives of millions of people suffering from organ failure, diabetes or some degenerative brain disorders could be vastly improved. This need has made xenotransplantation particularly attractive for biotechnology companies.

For most purposes, xenotransplantation will use pigs as the source of tissues and organs (Fishman *et al.*, 1998). There are ethical, safety and husbandry difficulties in exploiting primates, whereas pigs have large litters, grow to the appropriate size, and founder animals can be delivered by Caesarian section and reared in containment to maintain 'sterility'. Those religions that forbid eating pork have allowed the use of pig heart valves, which present no infectious hazard as they do not contain living cells. Other species, however, might be exploited for transplantation as well. The intracranial implantation of murine packaging cells delivering retroviral-mediated gene therapy to patients with glioblastoma is a form of xenotransplantation, though the practitioners have not regarded it as such.

Cloned piglets: Millie, Christa, Alexis, Carrel and Dotcom. Courtesy of PPL Therapeutics.

The greatest danger of transplanting cells or organs from pigs into humans is not so much the uncertain physiological compatibility of xenografts or the immunological hurdles yet to be overcome, but rather the potential hazard of zoonoses—animal-to-human infections. In theory, tissues and organs from specific pathogen-free pigs should be much 'cleaner' microbiologically than tissues from an 'off-the-street' human cadaver. The fear of xenotransplantation, however, is that of an unknown infectious agent in the animal organ that could infect the transplant recipient and spread to other humans. 'The risk is not just the patient who will probably die shortly afterwards,' Emanual Goldman from the New Jersey Medical School writes in the *British Medical Journal*. 'The stakes are much higher, because the entire human population is put at risk.' Before we dismiss such a scenario as far-fetched, let us recall the animal origins of new influenza strains, Ebola virus, variant Creutzfeldt–Jakob disease, and SIVcpz from chimpanzees, which ultimately became HIV-1.

Paul Herrling of Novartis epitomized the optimistic attitude in the xenotransplantation debate when he said, 'Domestic animals have transmitted infections to humans throughout history. The additional risk of a successful xenotransplantation might be minimal.' It is true that natural zoonoses occur, but they are rare events. Xenotransplantation is likely to increase the risk for several reasons. By bringing animal and human tissue into direct contact, the physical barrier of our skin and gut, the first line of defence against infectious agents, is circumvented. Furthermore, immunosuppressing the human recipient to avoid graft rejection makes it easier for animal viruses to overcome the host's immune system. Producing transgenic or knock-out animals to help to avoid graft rejection by 'humanizing' the tissues may impair the immune system's ability to discriminate friend from foe (Weiss, 1998).

To prevent zoonoses in the first place, the pigs used for human xenotransplantation can be kept in containment, and rigorously screened to eliminate known pathogens such as porcine influenza or cytomegalovirus. While containment breeding will eliminate most infectious agents, recontamination by porcine circovirus and porcine parvovirus is difficult to avoid. But the greatest threat is the novel viruses that lurk undiscovered in animal tissues. Several have recently been identified in pigs: a calicivirus related to human hepatitis E virus, a torovirus, the Nipah virus that has killed hundreds of people in Malaysia and Singapore, and the porcine endogenous retroviruses (PERV).

PERV in particular pose a serious problem as they are vertically transmitted and cannot be eradicated by containment breeding. Like many other retroviral genomes, they are transmitted as Mendelian genomes in the host's DNA (Patience *et al.*, 1997). Approximately 50 copies of integrated PERV are present in pig DNA. It will not be easy to determine which PERV genomes can turn into an infectious virus in humans, or to breed them out of the pig genome. Among the three envelope glycoprotein subgroups of PERV, at least two can infect human cells in culture via different cell surface receptors. These human-tropic PERV are released from porcine kidney cells, lymphocytes and endothelium, i.e. in tissues that are candidates for xenotransplantation.

Animal cellular therapies have already been used. Surgeons have transplanted porcine islets of Langerhans into patients with severe insulin-dependent diabetes mellitus. Patients with Parkinson's and Huntington's disease are being treated in phase I clinical trials with dopaminergic neurons from fetal pigs (Fink *et al.*, 2000). Extracorporeal perfusion of human blood over porcine hepatocytes can be used in cases of acute liver failure until the organ recovers or a human donor organ becomes available (Levy *et al.*, 2000).

Almost 200 patients have so far been exposed to living porcine tissue, for the treatment of burns, extracorporeal blood treatments, and transplantation of islet and neuron cells. The good news is that retrospective surveys have not revealed evidence of PERV infection *in vivo* (Paradis *et al.*, 1999). Nonetheless, cross-species infection by PERV has recently been demonstrated in guinea-pigs. And human tumour xenografts in mice occasionally become infected by murine xenotropic retroviruses.

Recent experience with xenotransplantation reminds us that the risk of infection by animal microbes has not been uppermost in the mind of every transplant surgeon

The recent experience with human xenotransplantation reminds us that the risk of infection by animal microbes has not been uppermost in the mind of every transplant surgeon. Although researchers at Novartis, exploring the safety of xenotransplantation, have provided valuable analysis of PERV infection (Paradis *et al.*, 1999), the company has neither looked for other porcine infections in these patients, nor counselled the physicians concerned to reconsider the practice of extracorporeal blood 'cleansing' through pig organs. To avoid an uncontrolled outbreak of PERV and other infections in the long run, close monitoring of xenograft recipients will need to be introduced. While sensitive molecular tools for PERV detection are in place, the ethics and procedures for life-long follow-up of patients and their intimate contacts still need to be elaborated.

Pig organs are lost through three mechanisms known as hyperacute rejection (HAR), acute vascular rejection and delayed rejection. HAR is due to the presence of natural human antibodies to a carbohydrate antigen, αGal, expressed on the endothelial cells of most mammalian species (Rother and Squinto, 1996). Humans and old-world primates are in effect natural knock-outs for the necessary galactosyl transferase gene and therefore produce αGal antibodies, which in conjunction with complement destroy the porcine vasculature.

At present, solid organs from pigs, which may have a higher risk of carrying unknown viruses than cellular therapies, cannot be transplanted into human patients. But it may be only a matter of time until whole animal organs become available for the transplantation surgeon. The cloning of five piglets by PPL Therapeutics scientists earlier this year (PPL press release, 2000) was widely seen as an important step towards generating stocks of genetically modified animals whose organs may not be subject to HAR in a human recipient. The company is now working to generate and clone knock-out animals lacking the transferase gene. Another possibility of avoiding HAR that has already been achieved is to express the human complement-regulating proteins CD55/DAF, CD46 and CD59 in transgenic pigs (Cozzi *et al.*, 2000). But such knock-out or transgenic animals may compound the infection hazards, because enveloped viruses including PERV may be protected from sensitivity to human complement. Furthermore, CD55, CD46 and CD59 happen to be receptors for viral pathogens (Weiss, 1998) and therefore may participate actively in viral infections. And although tissue taken from these animals would evade HAR and possibly acute vascular rejection (Wang *et al.*, 2000), delayed rejection would still have to be treated with immunosuppressive agents (Cozzi *et al.*, 2000), which increases infection hazards.

In the long run, xenotransplantation might become superfluous if researchers learn to grow tissue from human stem cells. But reconstituting whole organs will be much more difficult. Moreover this promising research is held hostage to abortion politics in the USA, and suffers from a wide range of differing and confusing regulations in Europe. Until these ethical and political problems are resolved, xenotransplantation will be pursued for the sake of patients who can only be helped by this procedure.

None of the virological foreboding may be of overriding concern to the patient waiting for a new kidney or liver. For him or her, the benefit of xenotransplantation will outweigh the risk. But the risk–benefit ratio changes with the spectre of onward transmission of a novel infection to the human population at large. Sensible risk assessment and long-term surveillance are necessary to minimize the risk of a new animal-borne epidemic. At least, let us look before we leap over the cliff, possessed like the Gadarene swine (Mark 5, 1–20).

Bach, F.H., Fishman, J.A., Daniels, N., Proimos, J., Anderson, B., Carpenter, C.B., Farrow, L., Robson, S.C. and Fineberg, H.V. (1998) Uncertainty in xenotransplantation: individual benefit versus collective risk. *Nature Med.*, **5**, 141–144.

Cozzi, E., Masroor, S., Soin, B., Vial, C. and White, D.J. (2000) Progress in xenotransplantation. *Clin. Nephrol.*, **4**, 13–18.

Fink, J.S. *et al.* (2000) Porcine xenografts in Parkinson's disease and Huntington's disease patients: preliminary results. *Cell Transplant*., **2**, 273–278.

Fishman, J., Sachs, D. and Shaikh, R. (eds) (1998) Xenotransplantation: scientific frontiers and public policy. *Ann. N Y Acad. Sci.*, **862**, 1–251.

Levy, M.F. *et al.* (2000) Liver allotransplantation after extracorporeal hepatic support with transgenic (hCD55/hCD59) porcine livers: clinical results and lack of pig-to-human transmission of the porcine endogenous retrovirus. *Transplantation*, **69**, 272–280.

Paradis, K., Langford, G., Long, Z., Heneine, W., Sandstrom, P., Switzer, W.M., Chapman, L.E., Lockey, C., Onions, D. and Otto, E. (1999) Search for cross-species transmission of porcine endogenous retrovirus in patients treated with living pig tissue. *Science*, **285**, 1236–1241.

Patience, C., Takeuchi, Y. and Weiss, R.A. (1997) Infection of human cells by an endogenous retrovirus of pigs. *Nature Med.*, **3**, 282–286.

Rother, R.P. and Squinto, S.P. (1996) The α-galactosyl epitope: a sugar coating that makes viruses and cells unpalatable. *Cell*, **86**, 185–188.

Wang, H., DeVries, M.E., Deng, S., Khandaker, M.H., Pickering, J.G., Chow, L.H., Garcia, B., Kelvin, D.J. and Zhong, R. (2000) The axis of interleukin 12 and γ interferon regulates acute vascular xenogeneic rejection. *Nature Med.*, **6**, 549–555.

Weiss, R.A. (1998) Transgenic pigs and virus adaptation. *Nature*, **391**, 327–328.

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Going to the roots of the stem cell debate

The ethical problems of using embryos for research • *by Dietmar Mieth*

In the minds of many people and the public press, the term 'stem cells' has become a magic password for entering a medical utopia where physicians will be able to overcome all human ailments once and for all. The hope for this 'brave new world' comes from tiny cells that are still undifferentiated but have the potential to become a variety of different cells. By directing their growth and development, biologists could potentially use them to grow therapeutic 'spare parts' to treat diabetes or Parkinson's disease or to heal paralysed persons—just to name a few uses of this technology. In the most extreme vision of this future, even aging and death could finally be defeated as failing organs would be replaced by new ones freshly grown from stem cells. Although these goals are not yet within reach, they have already triggered intense medical research and have drawn interest from the public and the bio-pharmaceutical industry.

But the glossy promises of stem cell research are overshadowed by serious ethical questions that result from the origin of these cells. Pluripotent stem cells cannot yet be generated from cell lines. They have to be taken from a human embryo at an early stage of development. At the moment, the most important sources are aborted or spare embryos left over from *in vitro* fertilization. It is this method of stem cell generation that has drawn most of the criticism. Medical treatments using stem cells are not yet available, so the actual dilemma is not their application but rather the direction that research should take since it needs these cells and consumes their source now. If we want to pursue medical research using embryonic stem cells, we have to face the problems that the extraction of these cells from a human embryo brings with it.

> Anyone who is not prepared to accept the cruelty of 'nature' as an ethically restrictive argument, should not use it as a normative argument for indifference either

The debate about the ethics of stem cell research has reached an international level, and has spurred on widespread concern about biomedical research in general. The failure of society to address and resolve these questions is reflected in the differences of interim regulations that have been adopted in various countries. In the USA, research that uses embryos cannot be financed with public funding. In the UK, research on embryos is currently limited to *in vitro* fertilization and pre-implantation diagnosis. Belgium has not yet adopted any regulations for the generation and use of stem cells. The Council of Europe has not decided on guidelines either: the supplementary protocol to the Convention on Biomedicine on the protection of the embryo has not yet been written. Things are happening, but the outcome of the ethical debate is still open.

Let us start the discussion about ethical concerns with the problems that arise from the physical removal of stem cells from a blastula. The first question is whether these cells themselves should be considered embryos because they are totipotent and can become 'anything'. Or should they be considered just as cells because they are still capable of a number of developments but not of developing into a fetus if they are implanted in a womb? If we agree that these cells have lost the ability to become a human being, then we can exclude them from the discussion about the protection of the embryo. And what about the embryos that are used for experiments? Can the removal of stem cells damage an embryo? Where experiments on embryos have been permitted and pursued, non-implantation has been seen as the logical decision, indeed as the ethical imperative because of the possibility that they might have been